EXHIBIT 2

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

EXAMINER:	R. ROSENBERGER	: ATTY DOCKET:	GK-BIO-292C2
APPLICANT(S):	E.W. STARK	: GROUP ART UNIT:	2505
SERIAL NO.:	08/385,073	: FILED	02/07/95

TITLE: METHOD AND APPARATUS FOR OPTICAL INTERACTANCE AND TRANSMITTANCE MEASUREMENTS

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Assistant Commissioner for Patents
Washington, DC 20231

DECLARATION UNDER 37 CFR §1.132

SIR:

I, Harry Shamoon, MD, hereby declare the following:

I

I have received the Bachelor of Arts Magna Cum Laude from Columbia College in 1970, and the Doctor of Medicine from the Yale University School of Medicine in 1974. Internship and Residency at the Bronx Municipal Hospital, Albert Einstein College of Medicine occupied me from 1974 to 1977. I was a postdoctoral fellow at the Endocrine section of the Yale University School of Medicine from 1977 to 1979. I am a Diplomate of the National Board of Medical Examiners and the American Board of Internal Medicine with certification in Endocrinology and Metabolism.

II

I have been employed by the Department of Medicine, Division of Endocrinology and Metabolism, Albert Einstein College of Medicine since 1979, most recently as Professor of Medicine and Director, Model Demonstration Unit, Einstein Diabetes Research and Training Center. Since 1980 I have been engaged in research in the field of diabetes with funding from the American Diabetes Association (ADA) and the National Institutes of Health (NIH). I am currently a principal investigator on the Epidemiology of Diabetes Interventions and Complications (follow-up of the Diabetes Control and Complications Trial cohort) and the Diabetes Prevention Program (NIDDM Prevention Trial).

III

I am familiar with prior art devices which perform measurements of blood glucose for the management of diabetes, and with the problems and shortcomings of the prior devices. All of the

medically accepted prior devices require the invasive acquisition of a blood sample in order to make the determination. The need for a painful skin puncture causes many patients to resist testing as frequently as is medically desirable and there is a significant risk of infection when devices are used by multiple individuals. In addition, simple, low cost glucose meters using reagent strips have been shown to be less accurate than desirable when used by the typical patient. Recently a non-invasive glucose measurement device based on near-infrared reflectance was submitted for approval by the U.S. Food and Drug Administration however it was rejected because the data indicated that the accuracy was insufficient.

IV

There has been a long felt need for a device which could make accurate in-vivo, non-invasive measurements of blood glucose for the improved management of diabetes. The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) sponsored the 10 year long Diabetes Control and Complications Trial which demonstrated that tight control of blood glucose levels could reduce the incidence of various complications of diabetes by 42% to 76% depending on the complication. The Food and Drug Administration has recognized the importance and value of in- vivo, non-invasive blood glucose measurements and indicated the need for accuracy such that 95% of the results fall within +/-20% of the true value. The medical, quality of life and economic benefits of such a device would be of inestimable value.

V

I have carefully reviewed the previous series of tests conducted by Dr. Michael Berelowitz at the State University of New York at Stony Brook using the device described and shown in US Patent Application No. 08/385,073. The clinical tests conducted with the device involved five volunteer normal subjects. The blood glucose level of each subject was increased from the fasting level by intravenous introduction of glucose. The above device was used at frequent intervals to obtain non-invasive in-vivo measurements of the subcutaneous interactance spectrum of the thumb in the visible and near-infrared spectral regions from 400 to 1700 nm wavelength. Simultaneously, blood samples were drawn and the glucose concentration determined by a standard laboratory instrument. These data were then used to generate chemometric calibrations relating the spectral data to the measured blood glucose levels using the partial least squares (PLS) method. The performance of these calibrations were validated by the method of cross-validation whereby the blood glucose level of each sample is predicted from the spectral data using a calibration developed not including that sample's spectral data. A total of approximately 500 spectral data runs consisting of over 1000 spectra each were collected during these tests.

I have also conducted and supervised tests on two subjects at the Albert Einstein College of Medicine utilizing the above device and improved control of the position and pressure applied to the thumb. The first subject was a Type I diabetic whose blood glucose level was normalized at the beginning of the protocol and then reduced from normal to a hypoglycemic state by controlled administration of insulin and glucose. After 6 1/2 hours the blood glucose was rapidly raised to

approximately 150 mg/dl by intravenous introduction of glucose. Non-invasive, in-vivo visible and near-infrared interactance spectra were obtained on the thumb using the above device. Sets of spectral data were collected at intervals of 2.5 minutes, each set consisting of 1200 spectra measured over a 40 second time span. The blood plasma glucose was accurately determined in triplicate every 5 minutes by a standard laboratory instrument. Approximately 150 sets of data were obtained covering the critical low blood glucose situation. Again, these data were used to generate chemometric calibrations by PLS and the predictive performance was determined by cross- validation.

The second subject was a normal individual whose blood glucose was at the fasting level at the beginning of the protocol. In order to avoid introduction of exogenous glucose, the subject ate a breakfast and a lunch to elevate the blood glucose. Noninvasive, in-vivo visible and near-infrared interactance spectra were obtained from the thumb using the above device. Sets of spectral data were collected at intervals of 2.5 minutes, each set consisting of 1200 spectra measured over a 40 second time span. The blood plasma glucose was accurately determined in triplicate every 5 minutes by a standard laboratory instrument. After approximately 45 minutes of fasting data, the subject ate breakfast and then, after 4.5 hours, lunch. Due to an instrument misadjustment, only 58 of the 143 sets of data collected were usable. Therefore, these data were combined with the data from the first subject and used to generate chemometric calibrations by PLS. The predictive performance was determined by cross-validation.

VI

The results of the tests performed with the device are shown below in Figures 1 thru 7 and their interpretation and significance are briefly discussed.

The first four protocols run at Stony Brook, which covered the blood glucose range from approximately 100 to 360 mg/dl, were encouraging but they demonstrated the need for more sets of spectral data from each protocol to adequately support the PLS chemometric calibration process. The fifth protocol (patient: Steven), which covered a blood glucose range of 53 to 315 mg/dl, was extended to over 8 hours in order to obtain more than 200 sets of spectral data for analysis (figure 1). A root mean square difference (RMSD) of 17.5 mg/dl was obtained. These data (figure 2) clearly indicate that the device can provide sufficient accuracy in the above-normal glucose range but improvement was needed in the critical range below 100 mg/dl.

The first protocol run at Einstein (patient: Annmarie) lasted 6 1/2 hours covering the blood glucose range from 53 to 118 mg/dl. Three additional plasma glucose determinations were made at 15 minute intervals after introduction of a bolus of intravenous glucose. The results (figure 3) prior to introduction of the bolus of glucose clearly meet the objective of 95% of the data being within +/- 20% of the reference value. An RMSD of 6.65 mg/dl was obtained. Eight outlier detected by spectral analysis prior to calibration were rejected (o's in figure 4). In practice, the instrument would automatically detect and not report values for these rejected data. The patient would be requested to make another determination.

An unexpected result occurred after the bolus of glucose was injected. The NIR blood glucose predictions remained in the 50-60 mg/dl range (▪'s in figure 4) for nearly an hour although the blood plasma glucose increased very rapidly. These data were eliminated from the calibration that is used for prediction as they were clearly anomalies in the data set. The delay in the response to increased plasma glucose is unexpectedly long. Based on the quality of the earlier results, it is believed that there is probably a physiological basis for this discrepancy rather than any error in the NIR data although the exact mechanism is presently unknown.. The NIR determinations reported here are based on the correlation of blood plasma glucose values with spectral data obtained from all the tissue in the optical path, including interstitial fluid and cells. These data suggest that an important delay in tissue glucose levels may go undetected by conventional blood testing methods. Thus NIR determinations using this device may provide additional insight into the kinetics of glucose transport and utilization in the body. Further studies are being conducted to evaluate the mechanism and medical value of this phenomenon.

Using the data from this first protocol, a comparison was made between the dual-ring device that is the subject of the present patent application and the prior-art single-ring devices by using the signal from only one source ring for calibration and cross-validation. Calibration using the inner ring as the source and central detection resulted in an RMSD of 7.11 while using the outer ring as the source and central detection produced an RMSD of 7.89 compared with the dual-ring RMSD of 6.65, a 7% and 19% degradation respectively. The prediction data from the inner ring only (figure 5) and the outer ring only (figure 6) compared to that of the dual-ring device (figure 3) show a significant increase in the number of measurements falling outside +/-20% error, particularly in the most critical low blood glucose range.

The second protocol run at Einstein (patient: David) lasted 6 hours covering the blood glucose range from 87 to 148 mg/dl. The results of the combined data from the first and second protocols (figure 7) show that only 5 of the 177 data points used exceed +/- 20% error, over 97% falling within these limits. An RMSD of 8.22 mg/dl was obtained. Based on spectral analysis, five outliers (o's in figure 8) were eliminated from the second data set prior to calibration.

Examining the results (figure 8) during the rapid rise in blood glucose from 100 to 140 mg/dl that was caused by the breakfast meal, there is no evidence of a lag in the NIR response compared to the Beckman blood glucose values. This tends to confirm that the unexpected lag in the prior data was due to causes other than the NIR measurement and therefore it may have medical significance.

In summary, I believe that the results of testing so far provide strong evidence for the expectation that NIR methods based on the device of U.S .patent application 08/385,073 can be applied to biomedical research and patient care, and could dramatically help in the treatment of diabetes. With the prevalence of diabetes increasing in the US to ~16 million by the end of the century, and considering that diabetes costs of over $100 billion per year account for 15% of our nation's health care budget, new treatment modalities could have enormous benefits.

I, Dr. Harry Shamoon, hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fines or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Harry Shamoon MD

Signature of Declarant

Nov 11, 1996.

Date

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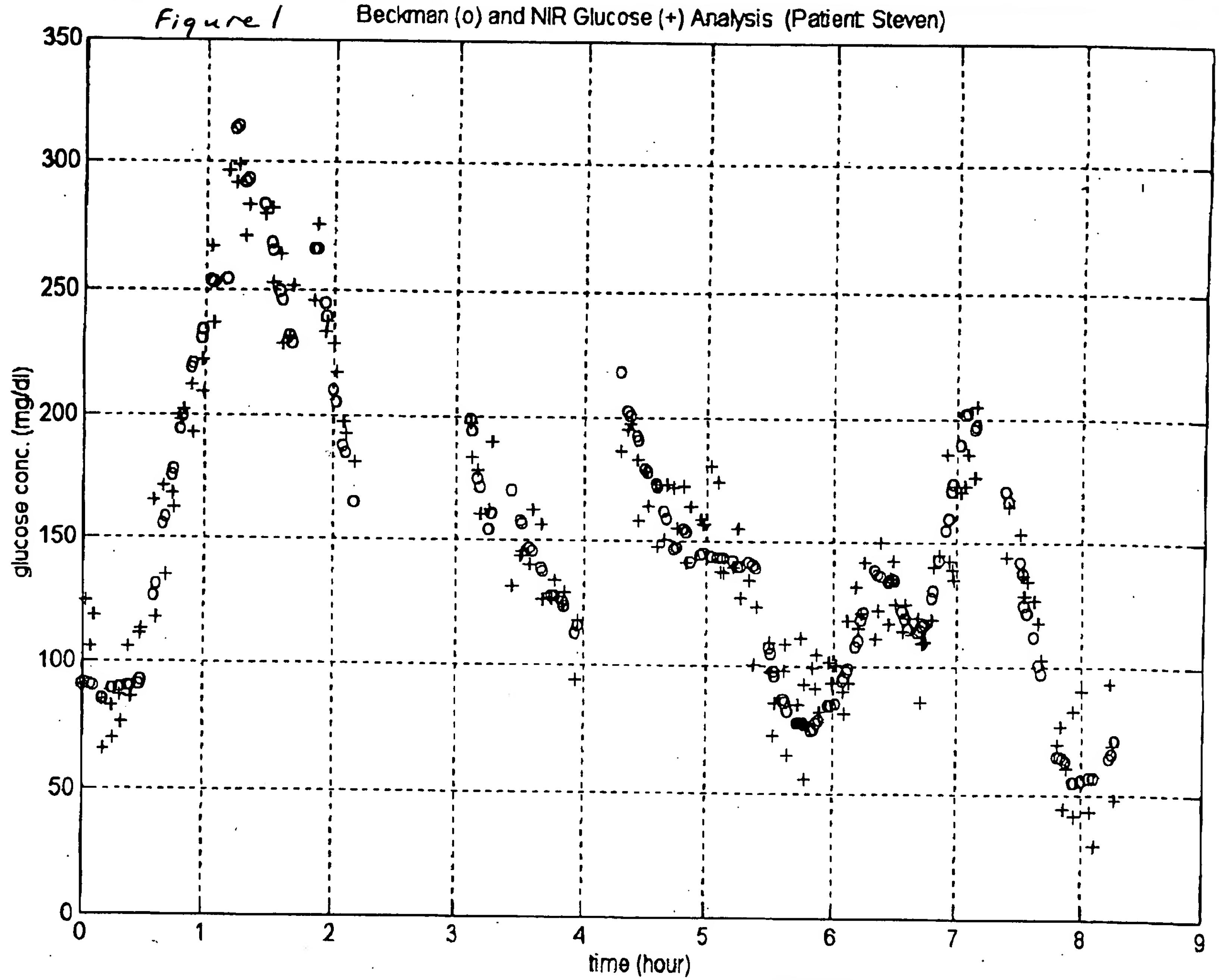
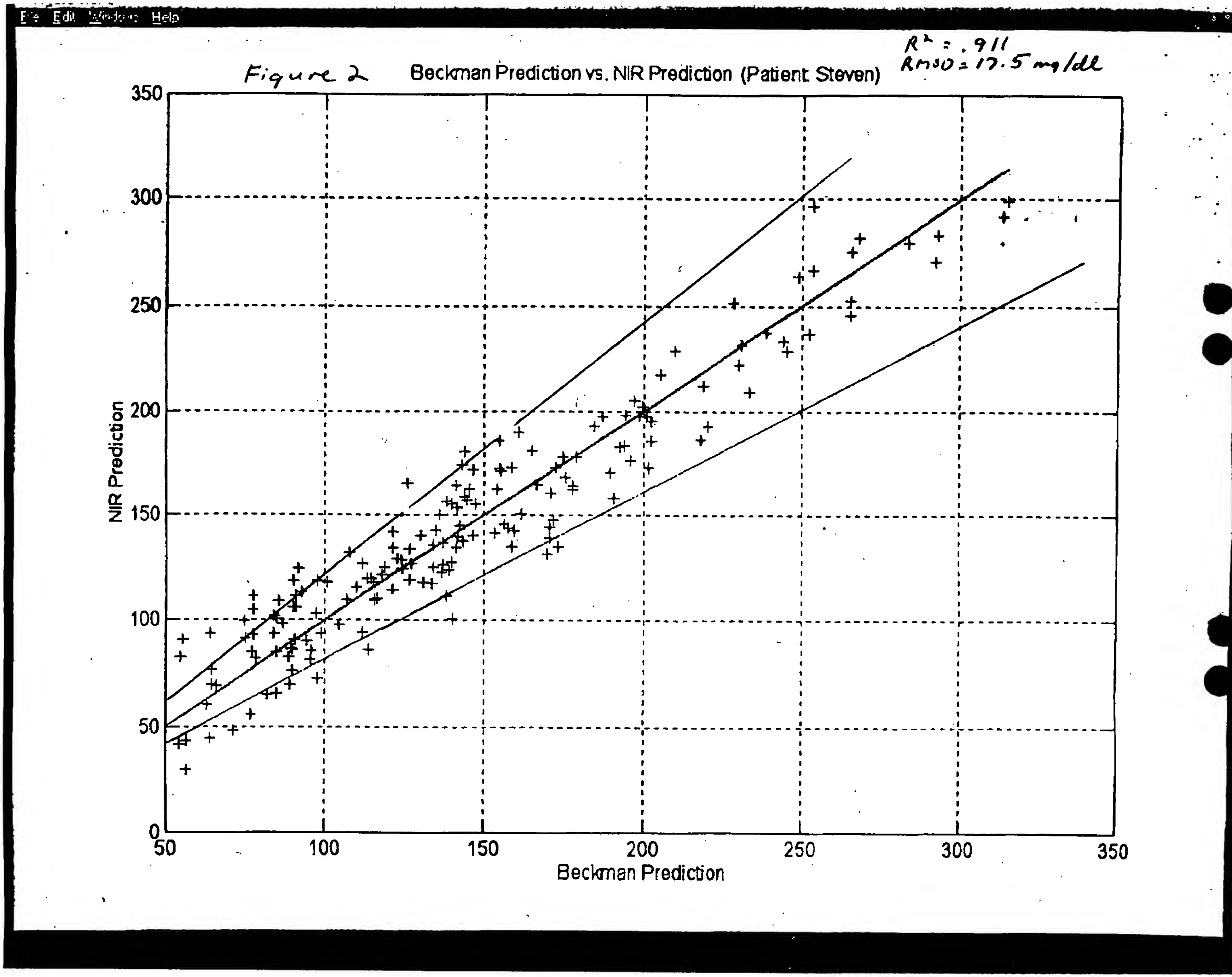
Figure 1



Beckman (o) and NIR Glucose (+) Analysis (Patient Steven)
glucose conc. (mg/dl)
350
300
250
200
150
100
50
0
0
1
2
3
4
5
6
7
8
9
time (hour)

Figure 2



File Edit Window Help
$R^2 = .911$
RMSD = 17.5 mg/dl
Beckman Prediction vs. NIR Prediction (Patient Steven)
NIR Prediction
Beckman Prediction
0
50
100
150
200
250
300
350

Figure 3

NIR predicted dual ring vs. Beckman, R-sqr=0.887, RMSD=6.65 mg/dl, factors=20, N=128 (patient: Annmarie)

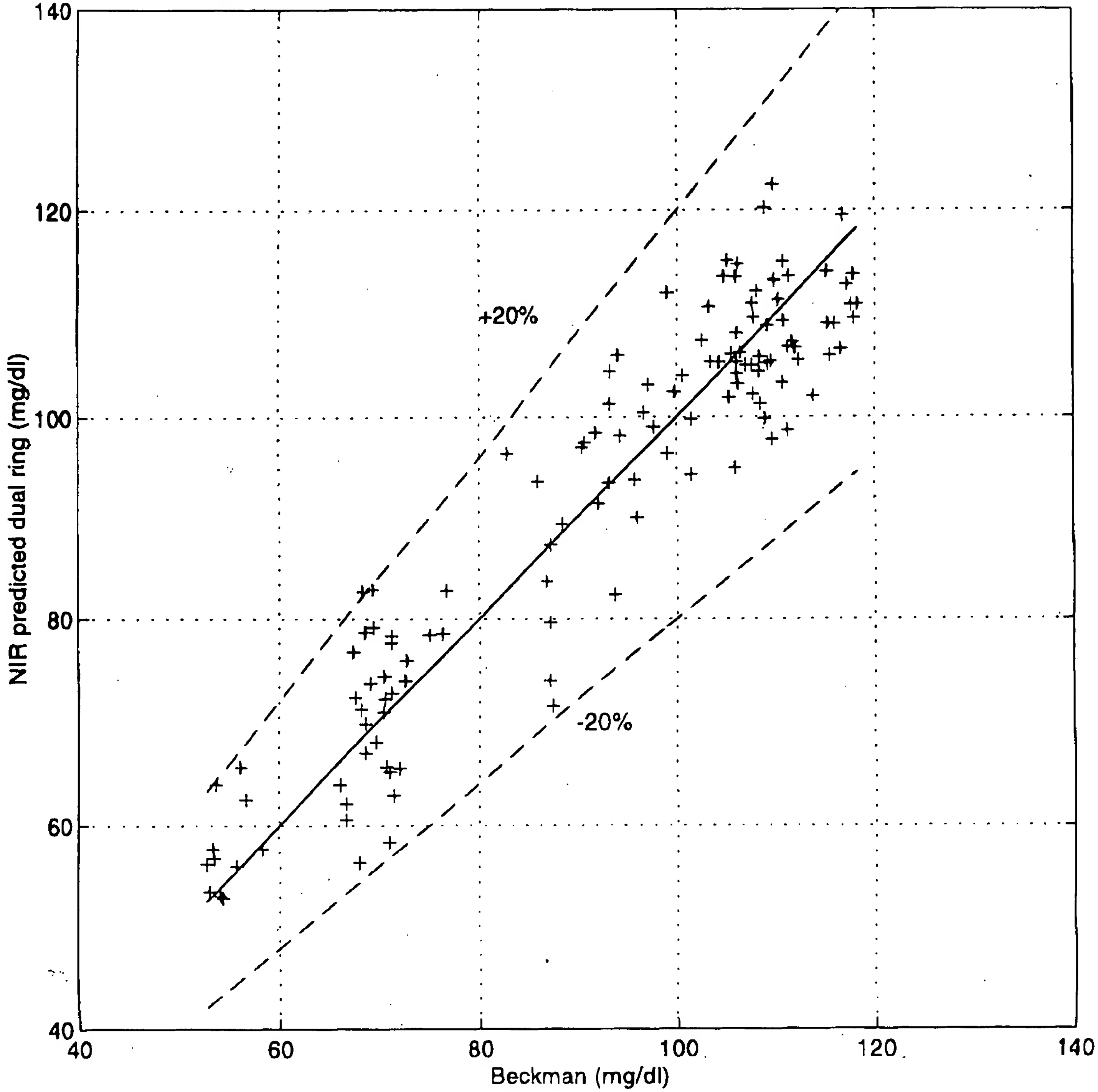


Figure 4

Beckman (-), NIR predicted (+), outliers (o), and anomalies (*) concentration v. time (patient: Annmarie)

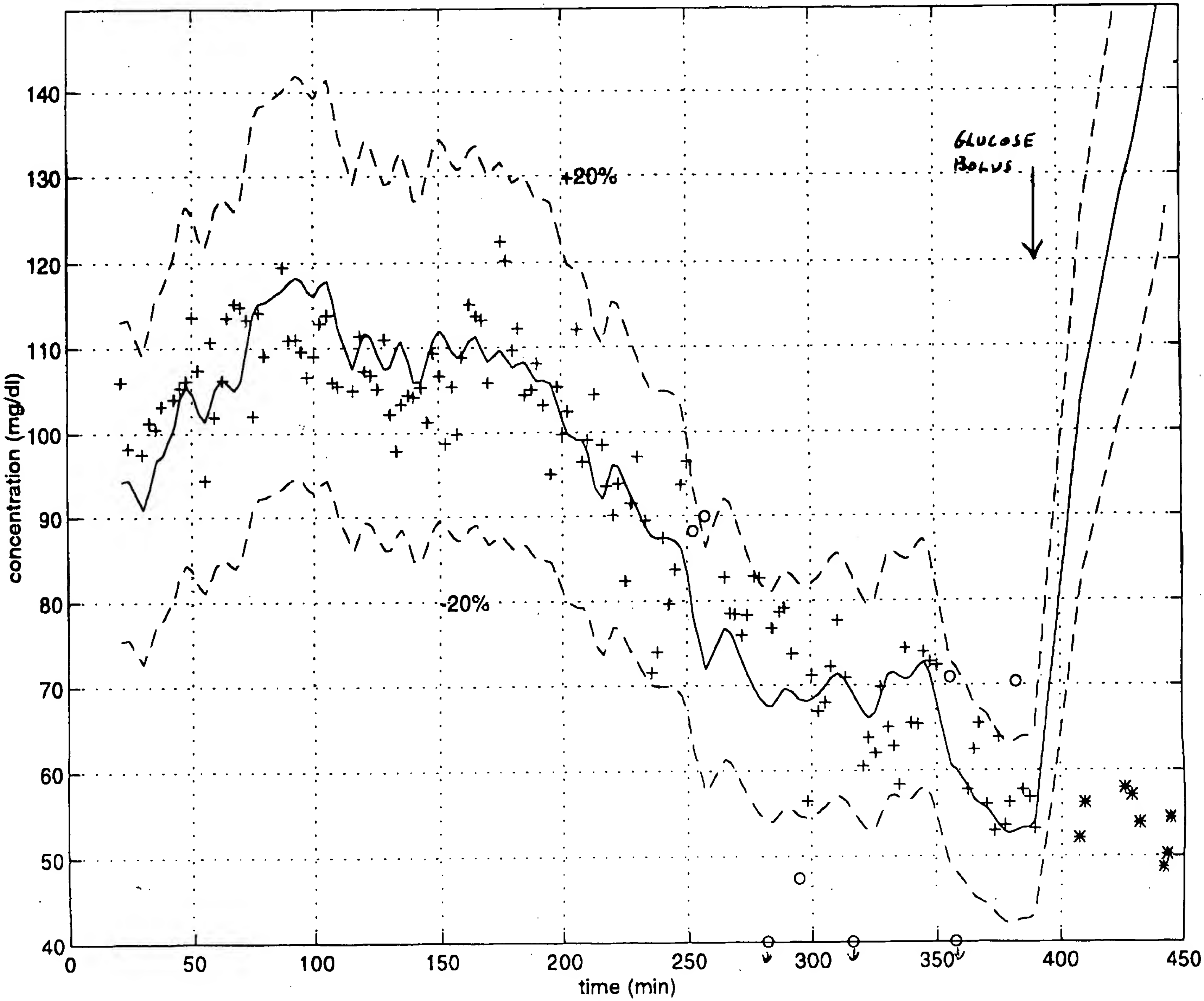


Figure 5

NIR predicted inner ring vs. Beckman, R-sqr=0.871, RMSD=7.11 mg/dl, factors=20, N=128 (patient: Annmarie)

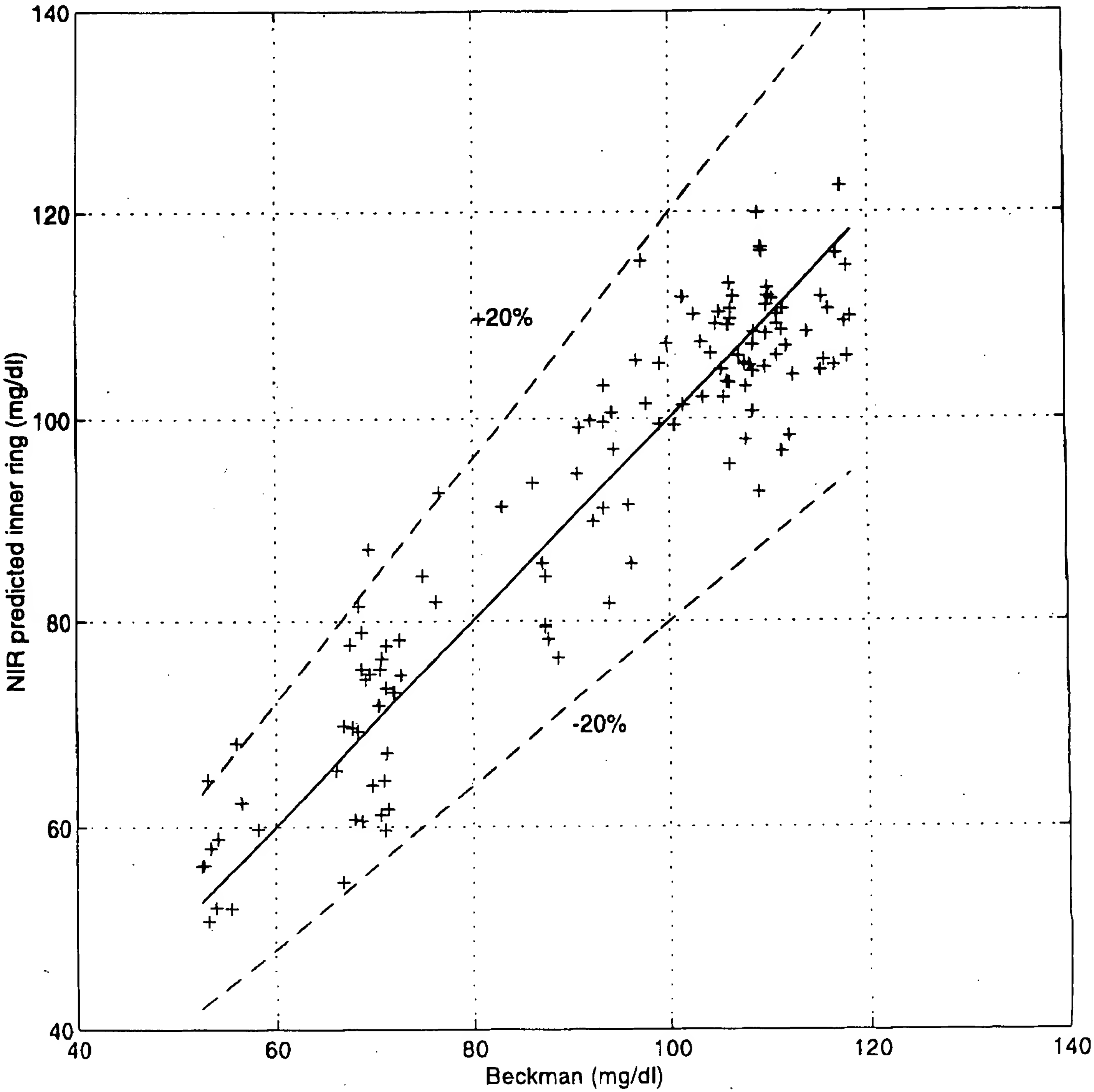


Figure 6

NIR predicted outer ring vs. Beckman, R-sqr=0.842, RMSD=7.89 mg/dl, factors=20, N=128 (patient: Annmarie)

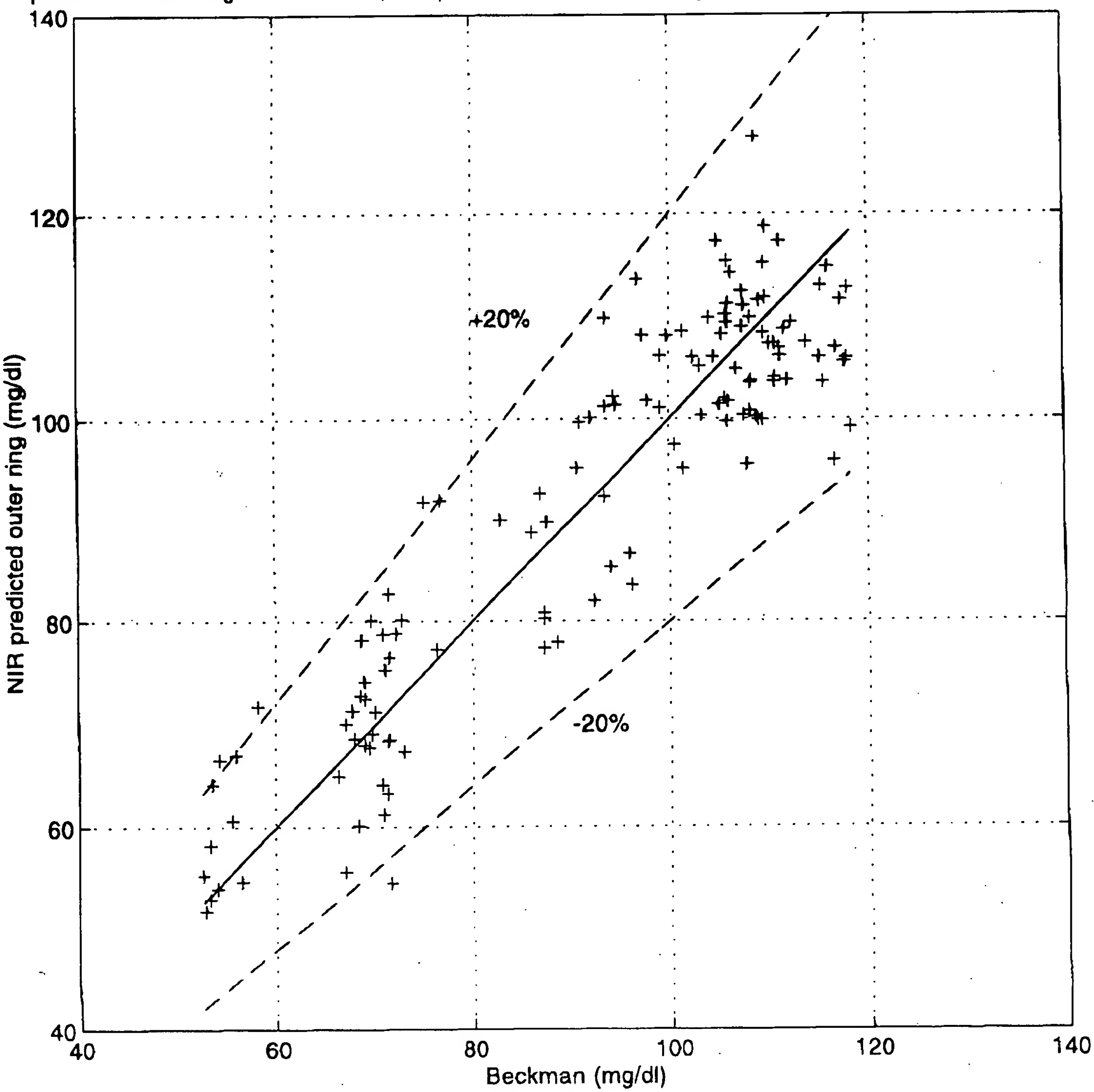


Figure 7

NIR predicted vs. Beckman, R-sqr=0.831, RMSD=8.59 (mg/dl), factors=24, N=177 (Annmarie and Dave Combined)

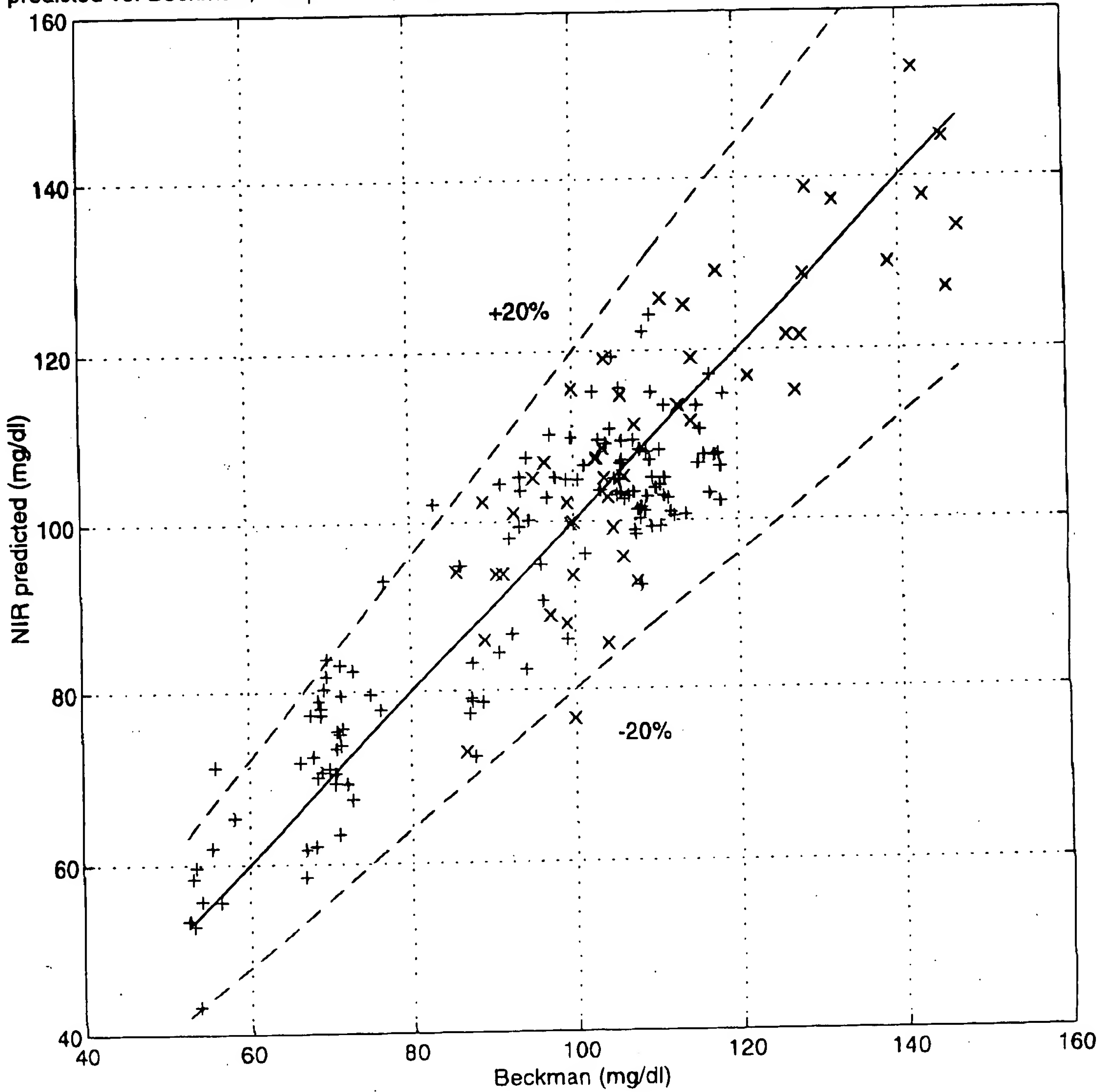
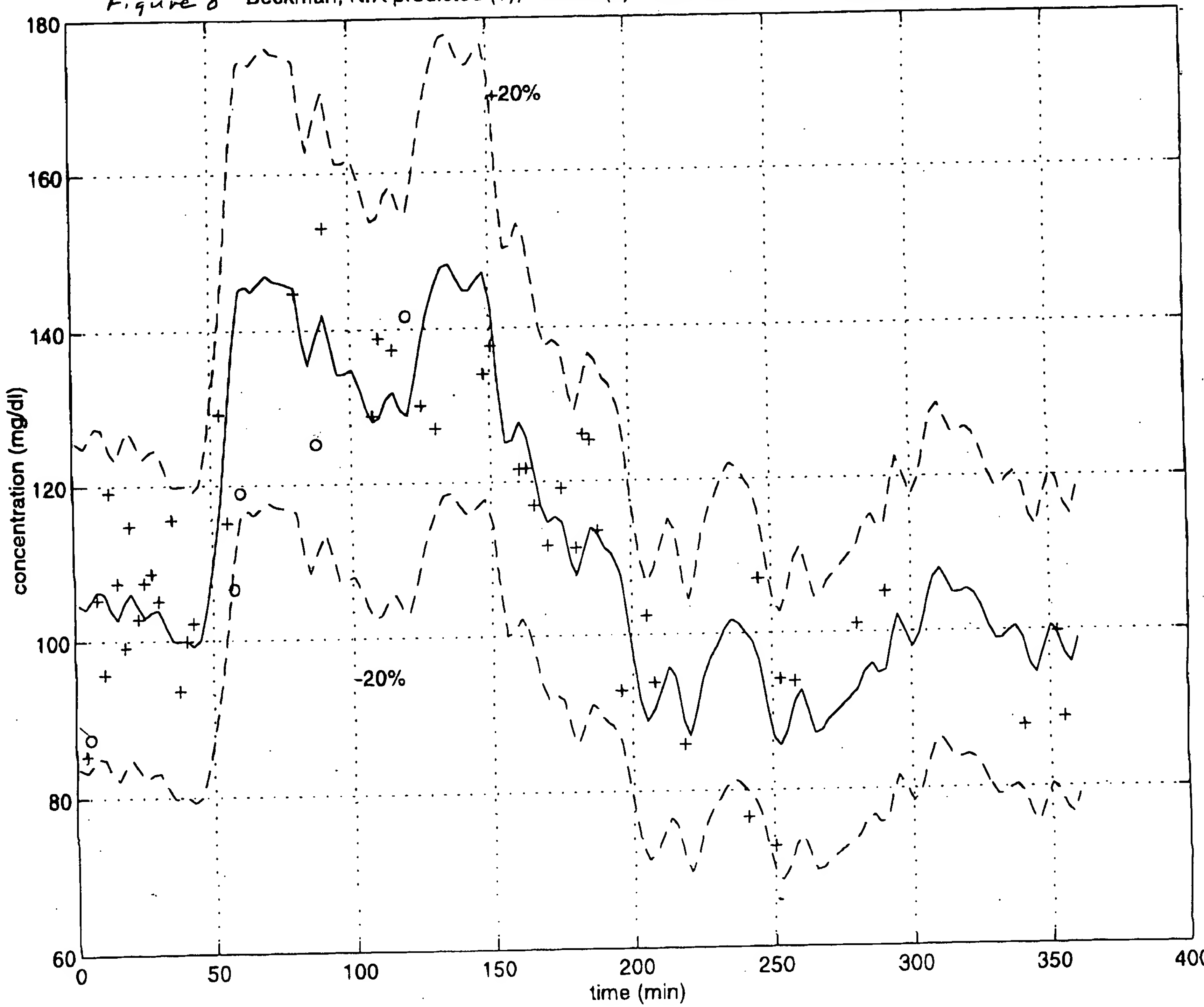


Figure 8 Beckman, NIR predicted (+), outliers (o) concentration vs. time (patient: Dave) Combined Calibration



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